REVIEW

Biomimetic design of materials and biomaterials inspired by the structure of nacre

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The micro-architecture of nacre (mother of pearl) has been classically illustrated as a ‘brick-and-mortar’ arrangement. It is clear now that hierarchical organization and other structural features play an important role in the amazing mechanical properties of this natural nanocomposite. The more important structural characteristics and mechanical properties of nacre are exposed as a base that has inspired scientists and engineers to develop biomimetic strategies that could be useful in areas such as materials science, biomaterials development and nanotechnology. A strong emphasis is given on the latest advances on the synthetic design and production of nacre-inspired materials and coatings, in particular to be used in biomedical applications.

Keywords: nacre; biomimetics; biomaterials

1. Introduction

Minerals have been produced by organisms of all five kingdoms for more than 3500 Myr, and are used for a variety of applications. Besides structural reasons, including protection, organism support, mobility or cutting and grinding, they may be applied for buoyancy proposes, as storage of ions or as optical, magnetic and gravity sensing (Mann 2001). The variety of shapes, microstructure, composition and functions are immense: for example, one may find nanocrystals in bone up to large crystals forming echinoderm spines; biogenic minerals may be found in either crystalline or amorphous forms; they can be combined with considerable amounts of organic materials, such as in bone, or almost without it, as in some mollusc shells; and the material may exhibit clear anisotropic or almost isotropic properties. Such diversity and the resulting properties have inspired many materials-oriented researchers in developing new materials and using biomimetic concepts for a variety of applications, especially to improve

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some mechanical properties of structural materials (Li & Kaplan 2003; Meldrum 2003; Mayer 2005; Barthelat 2007; Fratzl 2007). Some common features found in structural mineralized natural composites have provided clues for the design of new materials, which are as follows.

—*Hierarchical organization of the structure*. The biologically controlled growth of natural materials leads to highly multilevel architectures, exhibiting high control over the orientation of structural elements from the molecular level up to the final macroscopic structure (Fratzl & Weinkamer 2007). The different levels are held together by specific interactions between components and they operate in a synergistic fashion.

—*Mild-processing conditions*. The bottom-up approach for the production of biominerals is carried out through the self-assembling of elementary units under mild conditions of ambient temperature, neutral or physiological pH, always in an aqueous environment.

—*Recurrent use of molecular constituents*. Widely variable properties are attained from apparently similar elementary units. For example, calcium minerals, especially different forms of calcium carbonates, represent approximately 50 per cent of all known biogenic minerals and are produced by completely diverse living organisms (Addadi & Weiner 1992).

—*Energy saving*. Biology uses information and structure as the main vector for constructing materials, as compared with synthetic technologies that solve problems largely by manipulating usage of energy (Vincent et al. 2006).

—*Functionally graded properties*. The properties and composition along the material may vary, gradually or abruptly, adapting to the local requirements. A typical example, that will be discussed later, is the bilayer structure of some shells composed by nacreous aragonite and prismatic calcite (Weiner & Addadi 1997).

—*Self-healing and damage repair*. Some mineralized materials have the possibility to self-repair or remodel. For example, bone is continuously produced *in vivo* through the action of osteoblasts and absorbed by osteoclasts (Currey 2002) such dynamic replacement allows the continuous adaptation of the tissue to changing external conditions and to self-healing upon local injuries.

—*Multifunctionality*. Biocomposites may have other functions, besides the structural requirements. An example is bone that also acts as an ion reservoir and houses the haemopoietic stem cells in the bone marrow.

—*Durable interfaces between hard and soft materials*. Even in wet conditions, the organic components that link the mineral constituents exhibit interesting viscoelastic properties and fatigue performance.

—*Low density*. Structural natural materials exhibit densities that rarely exceed 3 g cm\(^{-3}\) (Wegst & Ashby 2004) and heavy elements, such as metals, are absent, whereas synthetic structural materials often have densities in the 4–10 g cm\(^{-3}\) range.

Depending on the complexity of the mineral formed, its biogenesis involves confinement in a space, ion pump control, construction of nucleation sites and control of orientation and morphology of the material. The mechanisms of biomineralization have been widely investigated, and elegant descriptions have
been provided, for example, by Weiner and Addadi (Addadi & Weiner 1992; Weiner & Addadi 1997). In many cases, it involves the deposition of the inorganic component in a very well-organized three-dimensional organic template, made of macromolecules, proteins or polysaccharides, typically rich in carboxylate groups and, in many cases, phosphate or sulphate groups. Such charged groups interact very well with mineral ions in solution or with the surface of the solid phase.

Mollusc shells are a particular case of mineralized materials and have been intensively studied as biomineralization models, especially bivalves and gastropods. Many different morphological types of shell structures may be found in such organisms. For example, in bivalve shells, it is possible to distinguish the simple prismatic, aragonite prismatic, nacreous, foliated, composite prismatic, crossed lamellar structure, complex crossed lamellar or homogeneous structure (Kobayashi & Samata 2006). Owing to the apparent simple morphology and peculiar properties, nacre has attracted considerable attention of biologists and materials scientists and engineers. Nacre is the inner lustrous mother of pearl layer of many shells, and pearls are also made of nacre. The basic structural motif in nacre is the assembly of oriented plate-like aragonite crystals with a ‘brick’ (CaCO₃ crystals) and ‘mortar’ (macromolecular component) organization. Many works recognized that such structure would be associated with the excellent mechanical properties of nacre, and biomimetic strategies have been proposed to produce new layered nanocomposites. This review evidences the more important characteristics of nacre, namely the structure and the mechanical properties, and focuses on the latest and more relevant attempts to produce new nacre-inspired materials, coatings and nanomaterials.

2. Structure of nacre

As in most structural natural materials, nacre exhibits a hierarchical structure (figure 1). The longitudinal cross section of nacre-containing shells, such as the abalone shell (figure 1a), exhibits two layers with distinct microstructures: a prismatic calcite layer (P) and an inner nacreous aragonite layer (N) (figure 1b). Such arrangement permits to optimize the protective function of the shell (Sarikaya & Aksay 1995): the outer layer prevents penetration of the shell, while the nacreous layer is capable of dissipating mechanical energy through inelastic dissipation. In the next hierarchical level, one can detect the so-called ‘growth bands’ (figure 1c). Such periodic growth arrests create mesolayers that can also play an important role in the mechanical performance of nacre, acting as powerful crack defectors (Menig et al. 2000). The thin organic layers separating the mesolayers are thought to be formed upon shell growth in periods of less calcification.

In a higher magnification level, one can detect that, in some cases such as in the abalone shell, the aragonite platelets are organized in columns, in which the intertablet boundaries form tessellated bands perpendicular to the lamellae boundaries (figure 1d); in other situations, such as in the pearl oyster, the intertablet boundaries are distributed randomly.
It should be noted that nacre is not the most common mineral organization of shells: the crossed lamellar structure is the most widespread structure in molluscs, in which the mineral lamellae are organized in more hierarchical levels (Pokroy & Zolotoyabko 2003); more information about more complex architecture may be seen elsewhere (Meyers et al. 2008). This structure may have consequences in the superior mechanical properties found in seashells owing to the enhanced ability for crack arrest at interlamellar boundaries across the different hierarchical levels (Kamat et al. 2000; Pokroy & Zolotoyabko 2003). Another aspect of seashells is the fact that the biocomposite should be used by a variety of different organisms of the same taxon and used in anatomically different environments. Therefore, the material should function under many different situations and should respond mechanically more or less equally from all different directions. In the particular case of the cross-lamellar structure, we may have highly anisotropic mineralized basic blocks, but the design strategy throughout the entire levels of structure tends to enhance, as much as possible, the isotropic character of the macroscopic material; such aspects can be found in other mineralized materials in nature (Weiner et al. 2000).

In a 1 μm level, we can observe the accumulation of polygonal tablets made of aragonite crystals (orthorhombic; CaCO$_3$), with a thickness of approximately 0.5 μm (figure 1f). Organic material is located inside and between these tablets, corresponding to a volume fraction of approximately 5 per cent. A fraction of this organic component corresponds to proteins that control different aspects of the shell formation process, including the synthesis of transient amorphous minerals.
the evolution to defined crystalline phases and the organization of the crystals in
the overall three-dimensional structure. It was shown that there is a close contact
between peptide chains and the inorganic fraction, and that this association is
mainly established through amino acids containing carboxylic groups (Metzler
et al. 2008). A broad discussion on the properties of such molluscan shell protein
was reviewed earlier (Marin et al. 2008).

Different models for the general structure of the organic fraction in nacre have
been proposed in the last 15 years. Careful cryo-transmission electron microscopy
experiments were reported earlier on demineralized nacre, where it was suggested
that the interlamellar sheets are mainly composed by highly oriented β-chitin
fibrils (Levi-Kalisman et al. 2001). The authors hypothesized that, next to such
fibrils, discontinuous layers (or patches) of aspartic acid-rich proteins might
be detected; the silk-like proteins should mainly be present within the
sheets and intracrystalline acidic glycoproteins would also be present within
the mineral tablets.

The mortar fraction in nacre may reveal a few nanoscale mineral columns
through the organic matrix layers (see arrow in figure 1f), randomly
distributed in the surface of the aragonite tablets. Such mineral bridges have
diameters of approximately 46 nm and a height of 26 nm (Song et al. 2003).
Moreover, the surface of the aragonite platelets is not smooth, but presents
nanoscale asperities that could also influence the mechanical properties of nacre
(Wang et al. 2001; figure 1e).

In a lower hierarchical level, the tablets are then composed by the assembly of
nanograins with an average size of 32 nm delimited by a fine three-dimensional
network of organic material (Li et al. 2004; Rousseau et al. 2005; figure 1g).
However, synchrotron spectromicroscopy and X-ray absorption near-edge
structure spectroscopy measurements suggest that individual tablets behave
as single crystals under polarized X-ray illumination, and the nanograins are all
co-oriented within each tablet (Metzler et al. 2007). The same authors showed
that the growth direction of such crystal is not oriented parallel to the c-axis of
the individual tablet and that there are columns of co-oriented tablets, with the
number of tablets per co-oriented column varying between 1 and 40. Such
observations are consistent with a model for nacre growth that includes the
formation of randomly distributed nucleation sites, preformed on organic matrix
layers before tablet nucleation and growth (Metzler et al. 2007). Such
information is important to elucidate the formation mechanism of individual
nacre tablets; as referred to by Qiao et al. (2008), three main hypotheses include
(i) single crystal growth, (ii) coherent aggregation of nanograins, and (iii) phase
transformation from amorphous carbonate or metastable vaterite. Such authors
monitored the growth process of nacre-like tablets in vitro and suggested a
multistep process, from the formation of an amorphous calcium carbonate layer,
an iso-oriented growth of nanostacks to the assembling into hexagonal tablets
(Qiao et al. 2008). The growth of consecutive aragonite platelets proceeds
through successive arrest of growth by means of a protein-mediated mechanism
followed by the reinitiating in a Christmas-tree pattern, as suggested by some
authors (Meyers et al. 2006, 2008). The organic fraction of nacre plays an
important role in the mineralization process; in fact, an organic scaffolding was
observed during the steady-state growth of tiled aragonite (Lin et al. 2008a).

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3. Mechanical performance

Considerable work has been carried out on the measurement of the mechanical properties of nacre. One of the first works with such extensive experiments was reported by Currey (1977) on a variety of bivalves, gastropods and cephalopods, where values of tensile, compressive and bending strength, modulus of elasticity and work of fracture were presented. The typical stress–strain curves present an elastic region followed by a plastic behaviour before failure. The important work of Jackson et al. (1988) reported values of elastic moduli of approximately 70 GPa (dry) and 60 GPa (wet) in nacre from the shell of a bivalve mollusc, *Pinctada imbricata*, and tensile strength of approximately 170 MPa (dry) and 130 MPa (wet). The effect of hydration is particularly important for the toughness of nacre. The work of fracture of dry nacre is approximately 350–450 J m$^{-2}$ and could go up to 1240 J m$^{-2}$ in wet conditions. The work of fracture in monolithic calcium carbonate was approximately 3000 times less than the ones measured in nacre. A decrease of this parameter upon drying crossed-lamellar seashells was also observed in another study (Neves & Mano 2005). This indicates that moisture has a significant plasticizing effect on the proteinaceous layers, thus leading to an increase in toughness.

In another study, standard tests performed on nacre determined a fracture toughness of $8 \pm 3$ MPa m$^{1/2}$; in four-point tests, and a fracture strength of $185 \pm 20$ MPa, in three-point bend tests (Sarikaya et al. 1990)—such values are better than most technical ceramics processed by conventional bulk techniques and are comparable with ceramic matrix (e.g. ZrO$_2$–Al$_2$O$_3$) and metal–matrix (WC–Co) composites; moreover, the results in nacre represents an eightfold increase in toughness over monolithic CaCO$_3$. Quasi-static and dynamic compression and three-point bending tests on abalone shells showed that the mechanical performance exhibited a strong orientation dependence in strength, as well as significant strain-rate sensitivity.

The main effect for the high toughening properties of nacre has been claimed to be the consequence of the highly tortuous fracture path occurring in the material with the exposing of the surface of ceramic platelets. However, the high amount of energy dissipation experimentally measured cannot be explained by just considering the simple brick-and-mortar architecture of nacre. Some more mechanisms able to contribute to energy dissipation in this material are presented as follows.

— *Organisation of the platelets and the organic fraction.* The architecture of nacre is such that if a stress normal to the platelet plane is applied, then the ductile organic fraction that glues the crystals will prevent an uncontrolled crack growth (Sumitomo et al. 2008). This viscoelastic dissipative process takes place mainly in the hydrated state, and dry nacre behaves very much similar to pure aragonite because the organic material becomes brittle (Barthelat 2007). The transition from a brittle (glassy) state to a more elastomeric behaviour was observed in a polysaccharide (chitosan) upon hydration using non-conventional dynamic mechanical analysis, in which a glass transition could be detected in intermediate hydration levels (Mano 2008; Caridade et al. 2009). Photoacoustic Fourier transform infrared spectroscopy results suggest that the water present at the nanograin interfaces also contributes significantly to the viscoelasticity of nacre (Mohanty et al. 2006; Verma et al. 2006). It was also suggested that proteins composing the organic matrix, such as Lustrin A, exhibit a highly modular
structure characterized by a multidomain architecture with folded modules and acts as a high-performance adhesive, binding the platelets together (Smith et al. 1999). Upon increasing stress, these biopolymers elongate in a stepwise manner as folded domains or loops are pulled open, with significant energy requirements to unfold each individual module—it was claimed that this modular elongation mechanism may contribute to the amazing toughness properties in nacre (Smith et al. 1999).

— Asperities onto the surface of the aragonite tablets. Wang et al. (2001) established that nacre exhibits inelastic deformation in both shear and tension. The stress, at which the inelastic deformation proceeds, is governed by the nanosized mineral asperities present on the surface of the tablets that act as an important source for shear resistance.

— Mineral bridges. Song et al. (2003) confirmed the existence of nanosized mineral bridges between the aragonite tablets, distributed among the organic matrix (figure 1). The existence of such inorganic bridges reinforces the weak interfaces, such that the interfaces become just suitable for the crack to extend in itself (Song et al. 2003).

— Interlocks. It was shown that the successive layers of aragonite are not simply set down one over the other, but that interlocking is present between platelets of nacre (Katti et al. 2005). Simulation studies showed that the existence of such interlocking has a significant role in the deformation behaviour and the progressive failure of interlocks helps in limiting catastrophic failure of nacre, contributing to the increase in toughness (Katti & Katti 2006).

— Waved surfaces. Although often described as flat, the aragonite tablets exhibit a rather convoluted surface, with a waviness that can reach up to half of its thickness in amplitude (Barthelat 2007). Upon stress, such non-flat topography progressively locks tablets sliding, rendering more difficult the separation of the tablets from their interfaces.

— Nanoscale nature and organization of the building blocks constituting nacre. The hierarchical structure found in many natural structural materials, such as bone, tooth and shells, is such that the smallest building blocks are generally on the nanometre length scale. It was elegantly demonstrated that such nanocomposites exhibit a generic mechanical structure in which the nanometre size of mineral particles is selected to ensure optimum strength and maximum tolerance of flaws (Gao et al. 2003). Moreover, based again on micromechanical analysis, it was shown that such layered systems, consisting of two materials with different elastic moduli (inorganic and organic components in nacre), may result in a shielding or anti-shielding effect to the crack tip, leading to a change of the crack driving force and energy consumed by the fracture process (Fratzl et al. 2007).

— Rotation and deformation of aragonite nanograins. The nanosized grains composing the aragonite tablets (figure 1) exhibit a ductile nature (Li et al. 2004). Such deformability of the aragonite platelets is relevant for the nacre’s fracture toughness. Very interesting in situ atomic force microscopy (AFM) observations of the nanogranular texture of the aragonite platelets during mechanical deformation allowed to conclude that, under this process, nanograin rotation and deformation occur, facilitated by the existence of the biopolymer spacing between the nanograins, which will contribute to energy dissipation in nacre (Li et al. 2006).
4. Nacre and bioactive materials for biomedical applications

Bioactive materials have the capability to develop a calcium phosphate layer in contact with bone upon implantation and typically exhibit a good integration with this tissue (Kokubo et al. 2003). Examples of bioactive glasses and ceramics are bioglass, sintered hydroxypatite and β-tricalcium phosphate, which were discussed in detail elsewhere (Kokubo et al. 2003). Strategies have been used to increase the osteoconductivity of non-bioactive materials, including biomimetic methodologies (Oliveira et al. 2003). It is also possible to prepare bioactive composites using biodegradable polymers, as reviewed by Boccaccini and co-workers (Rezman et al. 2006). Such composites may be modified in order to induce mineralization upon special external stimuli, such as temperature (Shi et al. 2007) or pH (Dias et al. 2008). Bioactive nanocomposites may be produced using nanosized bioactive particles. For example, glass-ceramic nanoparticles were produced through a sol-gel procedure, and used to fabricate biodegradable composites for tissue engineering applications (Hong et al. 2008, 2009). Such nanoparticles were also used in the production of bioactive coatings based on the nacre structure (see example in §5; Couto et al. 2009).

As in synthetic bioactive materials, it was shown that nacre itself integrates well into bone tissue (Atlan et al. 1999; Berland et al. 2005), and may stimulate the differentiation of stem cells into the osteoblast lineage (Rousseau et al. 2008; Zhu et al. 2008). It was shown that nacre coatings or seashells may be transformed into apatite using mild-condition chemical methodologies (Vecchio et al. 2007; Guo & Zhou 2008). Such strategies may be interesting if it is intended to keep the internal hierarchical structure of the natural composite. It is then clear that nacre, or nacre-based materials, may find applications in the biomedical field, namely in the orthopaedic or dental areas.

5. Synthetic strategies to produce nacre-based products

Lobmann (2007) presents a list of suggested terminology for processes related to biomineralization mimickism. Biomimetic materials synthesis refers to methods very close to nature, either using living organisms or using the respective materials isolated from organisms to prepare inorganic products. Bioinspired materials synthesis is used when concepts found in natural biomineralization are applied to the preparation of inorganic products using artificial materials. Finally, material bionics is related to the general imitation of structural features found in nature by any processing techniques.

Almost all methods related to the production of artificial nacre are based on laminates processing. Manne & Aksay (1997) reviewed some methodologies of producing nanolaminates, based on the use of inorganic particles. In the following sections, different methods, divided into four categories, will be analysed in more detail, and the more recent contributions will be highlighted. Special attention will be given to aqueous solution-based techniques at ambient or near-ambient temperature and pressure.
Covalent self-assembly or bottom-up approach

As mentioned previously, organisms adopt a bottom-up approach to produce well-defined hierarchical structures. The complex structures contain minerals with controlled size, shape, crystal orientation, polymorphic structure, defect texture and particle assembly (Verma et al. 2008). In the bottom-up methodology, an organic phase provides a template for inorganic crystals to nucleate and grow from supersaturated solution. This organic phase may cause acceleration or inhibition of crystal growth, depending on its functionality, molecular weight, concentration, density of functional groups on the backbone chain or side chain and whether the polymer is adsorbed on the surface or present in solution (Tsortos & Nancollas 2002).

With self-assembled monolayers, it is possible to obtain high densities of different functional groups as model surfaces for biologically inspired material synthesis. The preparation is as simple as adding a solution of the desired molecule onto the substrate surface and washing off the excess.

The formation of nacre involves use of organic macromolecules as templates for the nucleation of the minerals and control of the final material’s shape. It was shown that a nacre-mimetic architecture could be synthetically reproduced through an appropriate combination of inorganic crystals and organic polymers (Oaki & Imai 2005; Oaki et al. 2006). The specific interactions existing between the two components during crystallization of K$_2$SO$_4$ in the presence of poly(acrylic acid) generate the nanoscopic architecture (20 nm diameter blocks), and the switching between the modes of growth explains the formation of the macroscopic structure (microscopic nacre-like layered structure; Oaki & Imai 2005). Similar experiments were carried out with CaCO$_3$ and poly(acrylic acid), where it was seen that, as in real biominerals, the synthetic mineral was generated from bridged nanocrystals with incorporated organic polymers (Oaki et al. 2006). The role of polymers, both of biological and synthetic origin, with different functionalities has been investigated to understand fundamental principles governing biomineralization. Katti et al. (2008), exploring the bottom-up approach, synthesized composites of chitosan and polygalacturonic acid with hydroxyapatite by allowing precipitation of hydroxyapatite in the presence of biopolymers. The authors concluded that a significant improvement in elastic modulus, strain to failure and compressive strength has been achieved by incorporating polycationic and polyanionic biopolymers together with hydroxyapatite. Furthermore, structural analysis of these nanocomposites revealed a multilevel organization in nanocomposites. Another example of an approach based on a self-assembly process was proposed by Sellinger et al. (1998). The process starts with a solution of silicates, coupling agents, surfactants, organic monomers and initiators in a water/ethanol mixture; during dip-coating, micellar structures are formed and assemble into interfacially organized liquid-crystalline mesophases, thereby simultaneously organizing both the inorganic and organic precursors into the desired laminated structure. Organic polymerization, combined with continuous inorganic polymerization, lock in the nanocomposite morphology through covalent bonds within the organic–inorganic interface. Bonderer et al. (2008) produced clay containing nanocomposites, exhibiting excellent stiffness and strength. Thick hybrid films were produced based on a bottom-up colloidal assembly of strong
submicrometre-thick ceramic platelets within a ductile polymer matrix. Wei et al. (2007) have also used a bottom-up approach to obtain hybrid nanocomposites with alternated organic (diazo-resins and poly(acrylic acid)) and inorganic (CaCO$_3$ strata) layers, produced, respectively, by layer-by-layer and CO$_2$ diffusion methods.

Such bottom-up approaches may be useful in the design and synthesis of biomaterials for hard tissues, such as scaffolds for bone replacement or regeneration.

(b) Electrophoretic deposition

Electrophoretic deposition is a technique suitable for the production of nanocomposites in a low-cost approach that allows novel and complex material combinations. It involves the deposition of charged nanoparticles in suspension upon application of an external electric field, e.g. charge particles migrate to an oppositely charged electrode and deposit on it (Sarkar & Nicholson 1996; Boccaccini & Zhitomirsky 2002).

Wang et al. (2008) prepared poly(amic acid) (PAA)–clay nacre-like composite films by electrophoretic deposition of an emulsion of PAA, which was synthesized from pyromellitic dianhydride and 4,4’-dianminodiphenyl ether (ODA), containing various loadings of ODA-modified montmorillonite (MMT). MMT is included in the smectite mineral group, a monoclinic clay-like material. The structure of this layered silicate is based on very thin crystalline layers. The layers usually consist of a crystalline alumino- or magnesio-silicate, and the total sheet can be charged due to charge deficiencies when metal ions are exchanged for other types. Two tetrahedral layers (silicate sheet) surround one octahedral layer (gibbsite sheet), and the oxygen atoms are shared. MMT is the most used type of layered silicate in polymer nanocomposites because of the swellable layered structure (Brindley & Brown 1980; Akkapeddi 2000). The layered silicate was intercalated through reacting with PAA, achieving, in this way, an ordered layered assembly of the PAA–MMT composite film. The composite films displayed an ordered layered structure with improved thermal properties as well as stiffness (modulus increment in 155%) and strength (increment in 40%) when compared with those of the pure polymer film. Long et al. (2007) also produced films by electrophoretic deposition. The films of MMT, modified by acrylamide monomers, were produced in an aqueous suspension prepared by the dispersion of organic clay in distilled water, and followed by the electrophoretic deposition. A layered structure similar to those found in natural nacre was achieved with a hardness and modulus of the film of 0.95 and 16.9 GPa, respectively.

Lin et al. (2008b) used electrophoretic deposition combined with hydrothermal intercalation to prepare clay-modified electrodes with a uniform and continuous polymer/clay composite film of brick-and-mortar nanolaminated structure, mimicking nacre.

(c) Layer-by-layer methodologies

Layer-by-layer assembly is a simple and versatile method of producing thin films by alternately immersing a charged substrate into two solutions of oppositely charged polyelectrolytes. This technique is a suitable choice to
prepare nanostructured organic–inorganic films. A wide diversity of materials
may be employed, and film fabrication is performed under mild conditions, which
is particularly important for preserving activity of biomolecules (see Crespilho
et al. (2006) and references cited therein).

Tang et al. (2003) produced nanostructured organic–inorganic films in which
layers of more than 5 μm could be obtained. The multilayered films were
produced by sequential immersion of a glass slide into solutions of a polycation
and anionic MMT clay. The tensile strength of the prepared multilayers
approached that of nacre, whereas their ultimate Young’s modulus was similar
to that of lamellar bone. In a further investigation of these layer-by-layer (LBL)
assembly composites as potential bone implants, Podsiadlo et al. (2005)
demonstrated that, not only mechanical strength, but also antibacterial activity
can be introduced in these implantable materials by alternating clay layers with
starch-stabilized silver nanoparticles. In another work, the layer-by-layer
technique was combined with chemical bath deposition to prepare TiO2/organic
polymer multilayered films with a nacre-like architecture (Burghard et al. 2007).
The chemical bath deposition method operates at low temperatures (30–100 °C),
similar to the biomineralization process that occurs in natural organic–inorganic
nanocomposites. The authors took advantage of the negatively charged oxide
surface to alternate the TiO2 with positively charged polyelectrolytes. By using
this method, it was possible to achieve a film with a thickness of the order of a
few tens of nanometres. Osteoconductive glass-ceramic nanoparticles were
produced by a sol-gel methodology and showed to be bioactive when combined
with polymers (poly(l-lactic acid); Hong et al. 2008, 2009). Layer-by-layer
nanostructured hybrid coatings were also obtained by the sequential deposition
of a substrate in a suspension of bioactive glass-ceramic nanoparticles
(exhibiting a negatively charged surface) and a solution of a positively charged
polymer (chitosan) (Couto et al. 2009; figure 2). Such biodegradable coatings
were found to promote the deposition of apatite upon immersion in simulated
body fluid (figure 2d) and believed to have the potential to be used in a series of
orthopaedic applications.

Other methods proved to be faster than the dip-coating technique, as
described by Vertlib et al. (2008). Using a spin-coating layer-by-layer assembly
methodology, it was possible to prepare multilayered polyelectrolyte–clay
nanocomposites. This process is faster than the usual dip-coating method. For
example, the build-up of a 100-bilayer film with a thickness of approximately
330 nm needs less than 1 hour, which is 20 times faster than dip-coating processes
with the same characteristics. Another approach to the layer-by-layer assembly
technique was that from Podsiadlo et al. (2008), in which high-strength
and highly transparent nacre-like nanocomposites with poly(vinyl alcohol) and
Na+–MMT clay nanosheets were prepared. The formation of a high density of
weak bonding interactions between the polymer and the clay particles can lead to
high-strength nanocomposites.

The layer-by-layer assembly technique can easily produce thick nanocomposites
with pre-defined complex geometries, which is a clear advantage over the
other approaches mentioned above. On the other hand, the LBL process requires
many repeated steps to build up a film with practical thickness.

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Films can be obtained in solution, using soluble macromolecules anchored on insoluble matrices. This is called the template-inhibition strategy, and it consists of the mineral deposition from solution onto a well-ordered two-dimensional structure of a self-assembled film on solid or liquid substrates (Li & Kaplan 2003).

Kato et al. (Kato et al. 1998; Kato & Amamiya 1999; Hosoda & Kato 2001) successfully applied the template/inhibition strategy to develop thin-film crystals of CaCO₃ to chitosan (which acts as a solid matrix in the presence of PAA or poly(glycolic acid) as a soluble additive), cellulose and chitin films (on chitin fibres from CaCO₃ solution in the presence of an acid-rich macromolecule). The crystal growth from CaCO₃ solutions containing poly(acrylic acid) as a soluble acidic polymer results in the formation of CaCO₃ thin-film crystals approximately 0.8 mm in thickness on these solid matrixes. Layered polymer/calcium carbonate (CaCO₃) composite films were obtained by alternating spin coating of the polysaccharides and thin-film crystallization in the presence of an acidic polymer (Kato et al. 2000).

Sugawara & Kato (2000) proposed a synthetic approach for the fabrication of aragonite thin films by using a cooperative effect of Mg²⁺ and organic polymers with simple repeating units. The aragonite thin films of calcium carbonate were deposited on chitosan matrices with the cooperation of poly(aspartic acid) and magnesium ions in CaCO₃ solution, and their double-layered composite structures were obtained by alternating chitosan coating and thin-film crystallization. Such films resembled nacreous structures.

**Figure 2.** Bioactive glass nanoparticles (AFM image in (a)) were used to produce (b) multilayered coatings by (c) alternately dipping a substrate into a chitosan solution, water and nanoparticles suspension. (d) Upon immersion in simulated body fluid (SBF), an apatite layer could be detected on the surface. Data based on the results from Couto et al. (2009).
Xu et al. (1998) developed a mechanism for synthesizing ceramic thin film under mild conditions, providing new perspectives into the template-inhibitor–biomineral interaction. They synthesized macroscopic and continuous calcium carbonate thin films using the synergistic interplay between a semirigid template and a soluble inhibitor, two crucial controls used in natural biomineralization. The films were prepared at air–subphase interfaces by promoting mineral deposition with amphiphilic porphyrin templates, coupled with growth inhibition by the use of poly(acrylic acid) as a soluble inhibitor. The films produced had thickness ranging from 0.4 to 0.6 μm. Comparing films produced at 22 and 4°C, they concluded that, in the first case, a biphasic structure containing both amorphous and crystalline calcium carbonate was obtained and, in the second case, only a single amorphous could be observed.

(e) Other methodologies

To overcome some limitations in the techniques mentioned previously, some authors developed original strategies to transcribe the outstanding nacre-like designs into synthetic materials.

Deville et al. (2006), based on the fact that, during the formation of sea ice, the solutes presented in sea water are expelled from the forming ice and entrapped within channels between the ice crystals, prepared nacre-like architectures built with ceramic particles dispersed in water. First, layered materials were prepared through a freeze-casting method; the porous scaffolds were then filled with a second phase, e.g. an organic component, in order to produce a dense composite. This simple technique allows the production of layered composites with complex shapes and very interesting mechanical properties. The porous scaffolds obtained by this process exhibit strong similarity to the meso- and microstructures of the inorganic component of nacre.

Many other procedures are being developed towards the mimicking biomineralization process of natural nacre. An efficient biomimetic process to fabricate bulk-form-layered inorganic/organic nanocomposites was developed by Chen et al. (2008). The materials that the authors explored were: clay nanoplatelets as the mineral and polyimide as the organic constituency. Samples with a thickness of 10–200 μm were produced in a very short time using a centrifugal deposition process. This process resulted in an ordered nanostructure with alternating organic and inorganic layers. The mechanical properties were comparable with that of lamella bones, with a tensile strength of 70–80 MPa, Young’s modulus of 8–9 GPa and hardness of approximately 1–2 GPa.

Using a roll compaction technique laminated followed by hot-pressed sintering, Si₃N₄/BN composites were produced, imitating the layered microstructure found in nacre (Wang et al. 2000). The fracture surface of this material exhibits clear crack deflection and the fracture toughness was approximately 400 times higher than that of the monolithic counterpart. It would be much more interesting to fabricate nanolaminate structures under mild conditions, i.e. aqueous solutions and environmental temperatures and pressures. Moreover, it would be also important to have a good control of the organic–inorganic interface. Bennadji-Gridi et al., taking advantage of the specific behaviour of Na/Ca MMT in aqueous dispersion, prepared textured films by progressive evaporation of dispersions with low concentrations of delaminated
platelets. The increase in the concentration of ions during evaporation changes the nature of the clay mineral platelet face interactions from repulsive to attractive. After complete drying, a dense brick-like structure is obtained when a sodium salt is used as a deflocculant. The bending strength of the textured film is strongly affected by crack formation during drying, especially when the sample thickness increases (Bennadj-Gridi et al. 2006). Other laminated alumina/polyimide multilayer materials were fabricated by a dry process using sputtering and vapour deposition polymerization, which, under appropriate processing condition, could be free of cracks and delaminations (Naganuma et al. 2006).

In another different strategy, Chen et al. (2007) designed bioinspired composites based on microelectromechanical systems technology in order to mimic nacre structure.

Liu et al. (2008), in an attempt to simulate nacre, developed five different methods of arranging smectite clay tactoids. These tactoids, retaining sufficient rigidity for alignment, are able to provide ordered structures. The first step to prepare the nanocomposites was the dispersion of clay in water by ultrasonic agitation, followed by cleaning based on partial sedimentation. With the obtained suspension, a controlled phase separation was held by sedimentation, centrifugation, controlled rate slip casting, filtration and electrophoresis. The best results were obtained using the slip casting method, although all five methods led to well-aligned parallel layers of platelets. To produce the nanocomposites, polyethylene oxide was incorporated into the tactoids.

6. Conclusions

Hard biological materials, such as nacre, exhibit appealing hierarchical structural organizations and remarkable mechanical properties. Nacre and nacre-derived materials also show good interaction with bone, which makes this material attractive in the biomedical field. Bioinspired strategies have been presented to replicate some structural features of nacre—new materials and coatings with improved mechanical properties have been produced. However, the duplication of key features in artificial materials, such as the ability for self-repair and tenacity at interfaces, is still a challenge. More work is necessary to generate second-generation biomimetic composite materials with enhanced properties, using innovative multidisciplinary methodologies more close to the ones employed by Nature, including the use of synthetic biology concepts, improved techniques based on self-assembling or even combining cells or other biological elements in vitro in the materials fabrication.

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